Vital Role of Monocytes in Antifungal Killing

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Infection with the environmental fungus *Aspergillus fumigatus* can result in invasive aspergillosis (IA) in the lungs of immunocompromised patients, causing substantial morbidity and mortality. The innate immune defense against IA is known to involve neutrophils, but the role of monocytes in this process is poorly defined. A recent study published in *PLoS Pathogens* by the Dr. Tobias Hohl Lab (Memorial Sloan-Kettering Cancer Center, formerly of the Vaccine and Infectious Disease Division) examined the mechanisms of the antifungal activity of CCR2-expressing inflammatory monocytes ($CCR2^+$Mo). $CCR2^+$Mo cells were shown to increase the antifungal activity of neutrophils. They were also observed to exhibit their own antifungal effect via direct uptake and killing of the fungal conidia (spores).

IA can result in substantial mortality for patients with leukemia or who have received hematopoietic cell transplants but rarely affects immunocompetent patients. "Neutrophils have long been considered as the key player in defense against inhaled *A. fumigatus* conidia," explains lead author Dr. Anupam Jhingran. The researchers were interested in the role of $CCR2^+$ monocytes, however, and employed cell ablation strategies in mice to pinpoint their activity. "We found necessary function for monocytes in conidial clearance from the lung," he explains.

First, the researchers tested the effects that $CCR2^+$Mo cells had on the antifungal activity of neutrophils during infection with *A. fumigatus*. Although a lack of $CCR2^+$Mo cells did not result in reduced recruitment of neutrophils to the site of infection, neutrophil antifungal activity was impaired. The researchers used fluorescently labeled *A. fumigatus* conidia that emit different colors depending on whether they are alive or dead. They found that in $CCR2^+$Mo-ablated mice, neutrophils did not exhibit reduced ability to take up the conidia, but did have less conidial killing capacity. Thus, the $CCR2^+$Mo-mediated effect on the antifungal activity of neutrophils was linked to an influence on the direct killing of conidia by neutrophils.

Next, the researches wanted to look for any direct conidial killing by the $CCR2^+$ monocytes themselves. By using green fluorescent protein-expressing $CCR2^+$Mo cells, the researchers were able to detect the presence of either live or dead conidia inside the cells (see Figure). They found that, aside from assisting neutrophils in killing the fungus, the $CCR2^+$Mo cells also engulfed and killed conidia. Moreover, the rate that these cells killed the conidia was comparable to the conidial killing capacity of neutrophils.

Naturally, these results raise new questions. For example, it is not known how exactly monocytes inactivate conidia following their uptake. Additionally, the mechanism behind the effect of monocytes on neutrophils resulting in their enhanced antifungal activity needs further examination. Dr. Jhingran hypothesizes that, "soluble factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor (TNF) secreted by $CCR2^{+}$Mo act upon neutrophils to enhance their conidiacidal activity."

This research provides clear insight into the role of monocytes in immune system control of infections by *A. fumigatus*, which are 100% fatal if not treated. "These results are clinically relevant," explains Dr. Jhingran, "as a deeper understanding of anti-conidial mechanisms in monocytes could help devise immune enhancing strategies since monocytes can be harvested by leukopheresis, are relatively long-lived, and can be transferred to patients for potential therapeutic gain."

Espinosa V, Jhingran A, Dutta O, Kasahara S, Donnelly R, Du P, Rosenfeld J, Leiner I, Chen C-C, Ron Y, Hohl TM, Rivera A. 2014. Inflammatory monocytes orchestrate innate antifungal immunity in the lung. *PLoS Pathog* 10:e1003940.

See also: Jhingran A, Mar KB, Kumasaka DK, Knoblaugh SE, Ngo LY, Segal BH, Iwakura Y, Lowell CA, Hamerman JA, Lin X, Hohl TM. 2012. Tracing conidial fate and measuring host cell antifungal activity using a reporter of microbial viability in the lung. *Cell Rep* 2:1762-73

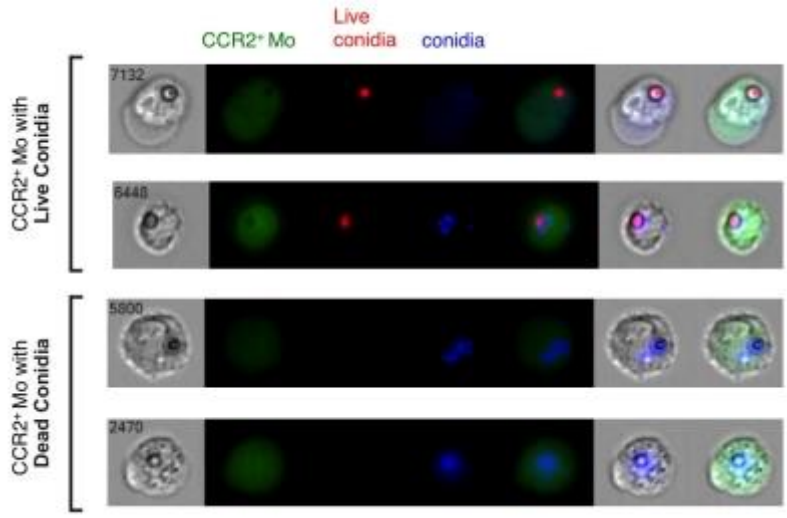

Image provided by Dr. Anupam Jhingran

Imaging cytometry of sorted lung CCR2+ monocytes (green) from a fluorescent-A. fumigatus-infected mouse. Conidia engulfed by monocytes were observed to be either alive (red) or dead (absence of red), indicating direct conidial killing by monocytes.